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Changes in endogenous gibberellin activity during winter dormancy in tea (*Camellia sinensis* (L.) O. Kuntze)

P.K. Nagar and Anil Kumar

Division of Biotechnology, Institute of Himalayan Bioresource Technology, Palampur - 176 061, H.P. India
fax: 91-1894-30433; e-mail: director@csihbt.ren.nic.in
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Abstract

Changes in gibberellin (GAs) activities were determined in tea shoots during winter dormancy and subsequent to dormancy release. Free GA-like activity was extremely low at the initiation of dormancy and remained so during the dormancy period. Conjugated GA-like activity (ficin hydrolyzable and β-glucosidase hydrolyzable compounds) remained high during the dormancy period. With an increase in free GA activity, conjugated GA activity decreased in tea shoots prior to dormancy release. The possible role of free and conjugated GAs in dormancy and following its release is discussed in relation to winter dormancy in tea shoots.

Introduction

Dormancy can be considered as a period of suppressed/suspended growth of a part or a whole plant. This phenomenon is viewed as a mechanism for species to survive adverse climatic conditions (Juntilla 1988). Because of its basic and applied nature (Leopold 1996), the regulation of dormancy has attracted considerable attention.

Tea is a perennial plant and, for production of commercial tea, the apical shoots are harvested several times at regular intervals during the period of active growth. Winter dormancy is common in all tea plants growing above 16° N or S latitudes where the photoperiod is less than 11 h 15 min during winter, with corresponding variations in day and night temperatures (Barua 1989). Such climatic conditions do not exist in the equatorial regions where tea plants grow throughout the year. The cessation of growth with the onset of winter dormancy and its resumption at bud break determines the state of growth or dormancy of tea shoots. Although external factors are considered to influence winter dormancy and its subsequent release, there is compelling evidence to suggest (Wareing 1977) that induction, maintenance and release of dormancy seems to be under hormonal control.

In a previous communication (Nagar 1996), high level of free abscisic acid (ABA) and phenols were detected in dormant tea shoots, and these decreased with dormancy release suggesting that these compounds could possibly play the main role during dormancy periods. Kakkar and Nagar (1997) discussed the possible roles of polyamines and other growth regulators in relation to winter dormancy in tea. Nandi *et al.* (1995) found GA_3 to be quite effective in inducing early bud break in tea. Investiga-

tions on the dynamics of endogenous gibberellins (GAs) in dormant organs are important for understanding the role of GAs in both dormancy induction or maintenance and in dormancy - breaking phenomena. In the present study we have analyzed free and conjugated GA-like activity in tea shoots during winter dormancy and subsequent to its release in an attempt to understand the relationship between them in this process.

Materials and methods

Plant material

Ten well-established bushes of China hybrid tea (*Camellia sinensis* (L.) O. Kuntze) were selected in the Institute's Tea Experimental Farm at Palampur (1290 m above msl, 32° 6' N, 76° 18' E) and from these 15 g of shoots were collected at 15 d intervals from the onset of dormancy to subsequent bud break *i.e.* from 30 October to 15 April as reported earlier (Nagar 1996).

Extraction and purification of GAs

Each sample of tea shoots (10.0 g F.wt.) was homogenized and extracted (3X) with chilled 80% methanol (1:3, w/v) containing butylated hydroxytoluene (100 $mg \cdot l^{-1}$) and the extracted residue was then removed. The extract was centrifuged (6,000 g, 20 min at 5 °C) to remove suspended materials. The supernatant was dried *in vacuo* and taken up in 0.1 M potassium phosphate buffer (pH 8.0). The buffer phase was acidified to pH 3.0 (1:1, v/v) with 2.0 M HCl and further extracted with water saturated ethyl acetate (3X). The ethyl acetate fractions were evaporated to dryness *in vacuo* and dissolved in 2.5 ml of the buffer (pH 8.0) for further analysis.

The acidic fraction was applied to a PVP column (20 X 2 cm) and eluted with four bed volumes of 0.1 M potassium phosphate buffer (Glenn *et al.* 1972). The eluate was evaporated to dryness, adjusted to pH 3.0 and extracted (3X) with water - saturated ethyl acetate (1:1, v/v). The ethyl acetate fractions were pooled, evaporated to minimum volume and loaded on a column (20 X 2 cm.) of charcoal:celite (1:2 w/w) for further purification. Acetone (250 ml) was passed through the column, the eluate was evaporated *in vacuo* to dryness and the residue taken up in methanol (HPLC grade) for analysis of free GAs.

HPLC analysis

For reverse phase HPLC, the equipment and configuration was described elsewhere (Nagar 1996). The samples were injected into a 20 µl injector loop and the elution was carried out with a linear gradient of 20-100 % methanol in 30 min at a flow rate of 1.5 $ml \cdot min^{-1}$. Column eluates were monitored with an online Kontron detector (D 430) set at 215 nm. HPLC fractions were collected each min, dried *in vacuo* and dissolved in methanol. Each HPLC fraction was bioassayed by the lettuce hypocotyl test (Frankland and Wareings 1960) with the following modifications. Ten lettuce seedlings (cv. Grand Rapids) that germinated for 48 h in darkness at 24±1 °C, having 0.8-1.0 cm long radicals were selected. These were placed in each test vessel (2.5 cm diameter glass vessel) on top of a filter paper disc (2.5 cm diameter) containing dried HPLC fraction and 1.25 ml of glass distlled water. The vials were placed under fluorescent lamps (Phillips) with an irradiance of 40 $W \cdot m^{-2}$. Hypocotyl lengths were measured to the nearest mm on the third day. The bioactive fractions were pooled and GA-like activity was estimated in terms of µg GA_3 equivalent$\cdot g^{-1}$ fresh mass.

Conjugated GAs

The residue left over after methanol extraction was air dried and treated with 1 % (w/v) ficin (Sigma, USA) in 0.1 M (pH 7.2) Tris-HCl (Jones 1964) to yield GA released from peptides and proteins termed as ficin hydrolysable form.

The aqueous fraction left over after ethyl acetate partitioning was extracted (3X) with *n*-butanol (1:0.5, v/v) according to Sembdner *et al.*(1970). The solvent was evaporated and the residue dissolved in 0.15 M Na-acetate buffer (pH 5.2) containing 0.1% (w/v) β-glucosidase (Sigma, USA) to yield GAs conjugated with sugars termed as the β-glucosidase hydrolysable form.

Both the enzymatic hydrolyses were separately carried out at 37 °C for 15-16 h with occasional shaking. The released free GAs were extracted with ethyl acetate from the hydrolysed supernatant,

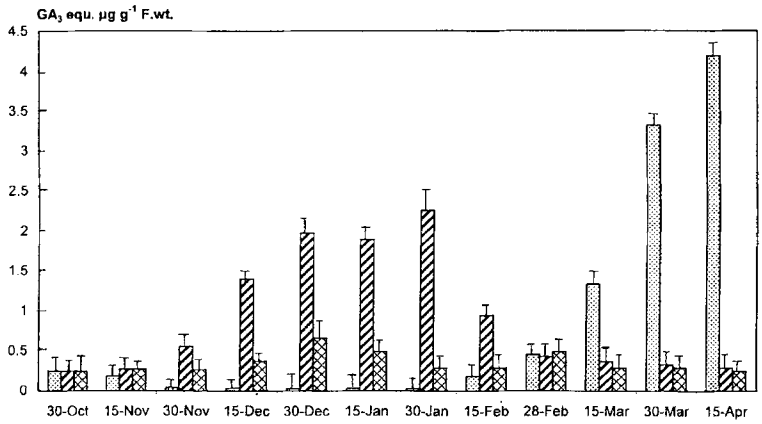

Fig. 1. Changes in the levels of free and conjugated GAs activity in tea shoots at 15 days intervals from the onset of dormancy (30 October) to subsequent to dormancy release (15 April). "dots" - free Gas, "lines" β-glucosidase hydrolysable and "check" - ficin hydrolysable, conjugates. SE (n=3) for each sampling are given as vertical bars.

acidified to pH 3.0 with 2.0 M HCl and purified as above. From these, GA-like activity was estimated after HPLC as done for free GAs.

Results

Changes in the level of free GA-like activity as well as in the levels of the GA released as a result of hydrolysis with ficin and β-glucosidase during winter dormancy and subsequent to its release in tea shoots are presented in Fig. 1. With the onset of dormancy (November onwards), free GA levels were extremely low even up to January 30. However, the levels increased after this period and reached their highest value (4.193 $\mu g \cdot g^{-1}$ fresh mass) on April 15, after dormancy release.

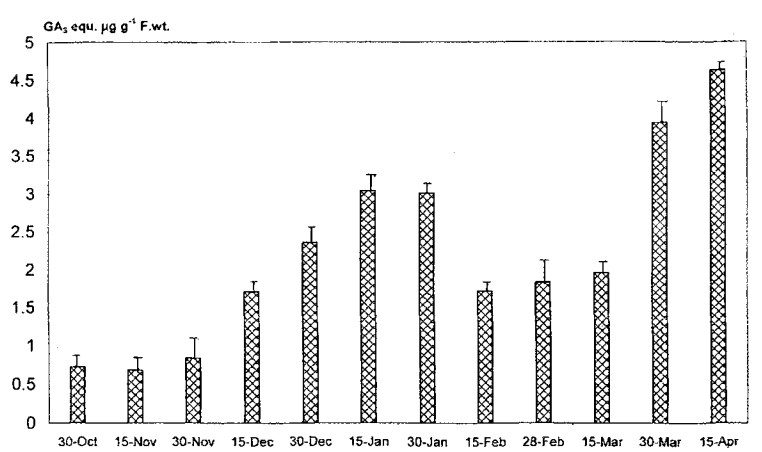

Fig. 2. Changes in the total gibberellins activity in tea shoots at 15 days intervals from the onset of dormancy to subsequent to dormancy release. SE (n=3) for each sampling are given as vertical bars.

Contrary to the very low levels of free GA-like activity during dormancy, conjugated forms of GAs were detected in appreciable amount during this period. In the case of β-glucosidase-hydrolysable forms (conjugated to sugars), the increase in the level was much greater than ficin hydrolysable form (conjugated to proteins and peptides). The levels of the former increased abruptly from December 15 and reached their highest value on January 15. The level of the ficin hydrolysable form also increased, but much more slowly, and were comparably low during this period. Overall, GA activity of the β-glucosidase - hydrolysable form was greater than the ficin hydrolysable form during the dormancy period. Both these fractions declined in their activity after the middle of February, whereas free GA activity increased.

Total GA activity was maximum subsequent to dormancy release (Fig. 2) as represented by all the three forms of GAs, but free GA activity predominated. Although total GA content was also high from December 30 to January 30, it represents mainly the ficin and β-glucosidase- hydrolysable forms.

Discussion

During winter dormancy shoot growth in tea is negligible. Subsequent to dormancy release shoot growth starts from the middle of March (Nandi and Palni 1993), coinciding with tea plucking in this area, the period during which free GA activity increased appreciably (Fig. 1). Exogenous application of GA_3 was quite effective in inducing early bud break (Nandi *et. al.* 1995), which supports the concept that GAs are involved in the dormancy process and its release. It is well known that dormancy can be broken by application of GAs in many plant species (Powell 1987, Luna *et al.*

1990). GA_3 is a known inducer of shoot growth and, in certain cases, it has been demonstrated that with a decrease in ABA levels during dormancy release, gibberellin levels increase (De Bottini *et al.* 1982, Talla 1989). ABA plays an important inductive role in the early stages of dormancy in tea shoots and during later stages some kind of promoting force becomes dominant to override the possible effect of ABA (Nagar 1996). The significant increase in free GA levels (Fig. 1) before dormancy release (first week of March) suggests that these compounds may be involved in dormancy release process. Thus in tea an inverse relationship between ABA and free GAs occurs during dormancy breaking process. The basic framework of the hormone theory of dormancy suggests that dormancy and its release depend on the interaction between naturally-occurring growth inhibiting and growth promoting substances. The interaction has often been described as a balance between simultaneously occurring growth promotive hormones like GAs and cytokinins versus the inhibitory substances such as ABA (Luna *et al.* 1990).

The decrease in both ficin - and β- glucosidase hydrolysable GA activity during the later stages of dormancy (February onwards) with an increase in free GA activity (Fig. 1) is suggestive of their metabolic interconversions. Conjugation is an important part of GA metabolism during all developmental plant processes (Schneider and Schllemann 1994) and the conjugated GAs, which are considered to be storage forms (Lenton and Appleford 1991, Schneider *et al.* 1992) will only be hydrolysed to their free pools and utilised as and when required. The formation and breakdown of GA conjugates presents one possible mechanism which determines free GA levels (Schneider and Schllemann 1994). In the present study a reasonable equivalence between the increase in activity of free GAs and the decrease in the activity of GA conjugates and *vice versa* indicates that this mechanism may be active. The equivalent changes in free and conjugated GAs in *Corylus* (Arias *et al.* 1976) and apple (Halinska and Lewak 1987) seeds is suggestive of the formation and hydrolysis of conjugates in the control of physiologically active levels of free GAs.

The results and interpretations presented in this paper indicate important and differentiated roles for endogenous GAs in the dormancy release phenomena of tea shoots. The results also indicate the involvement of a reversible formation of GA conjugates in the determination of free GAs levels. This supports the role of conjugated GAs as a reserve form of this important plant growth hormone.

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References

Arias I. William, P.M. and Bradbeer J.W. 1976. Studies in seed dormancy. IX. The role of gibberellin biosynthesis and the release of bound gibberellins in post-chilling accumulation of gibberelins in seeds of *Corylus avellana* L. Planta 131: 135-139.

Barua D.N. 1989. Science and Practice in Tea culture. Tea Research Association, Calcutta pp. 509.

De Bottini G.A., Bottini R. and Tizio R. 1982. Physiology of dormancy in potato tubers as related to endogenous regulators. Phyton 42: 115-121.

Frankland B. and Wareing P.F. 1960. Effect of gibberellic acid on hypocotyl growth of lettuce seedlings. Nature 185 : 255-256.

Glenn J.L. Kuo C.C. Durley R.C. and Pharis R.P. 1972. Use of insoluble polyvinylpyrrolidone for purification of plant extracts and chromatography of plant hormones. Phytochem. 11: 345-351.

Halinska, A. and Lewak S.T. 1987. Free and conjugated gibberellins in dormancy and germination of apple seeds. Physiol. Plant. 69: 523-530.

Jones D.P. 1964. Examination of the gibberellins of *Zea mays* and *Phaseolus vulgaris* using thin layer chromatography. Nature 202: 1309-1310.

Juntilla O. 1988. To be or not to be bud dormant: Some comments on the new dormancy nomenclature. Hort Sci. 23: 805-806.

Kakkar R.K. and Nagar P.K. 1997. Distribution and changes in endogenous polyamines during winter dormancy in tea (*Camellia sinensis* (L) O. Kuntze). J Plant Physiol. 151 : 63-67.

Leopold A.C. 1996. Natural history of seed dormancy. In: Plant dormancy, (Ed. Lang G.A.), CAB International, U.K. 3-16.

Lenton J.R. and Appleford N.E.J. 1991. Gibberellin production and action during germination of wheat. In: Gibberellins, (Eds. Takahashi, N., Phinney, B.O. and MacMillan, J.) New York: Springer 125-135.

Luna V., Lorenzo E., Reinesa H., Tordable M.C., Abdala G., Pharis R.P., and Bottini R. 1990. Dormancy in peach (*Prunus persica* L.) flower buds. I. Floral morphogenesis and endogenous GAs at the end of dormant period. Plant Physiol. 93: 20-25.

Nagar P.K. 1996. Changes in endogenous abscisic acid and phenols during winter dormancy in tea (*Camellia sinensis* (L.) O. Kuntze). Acta Physiol. Plant. 18: 33-38.

Nandi S.K. Palni L.M.S. and Rashmi 1995. Chemical manipulation of dormancy in tea shoots and associated biochemical changes. J. Plantn. Crops. 23 : 52-58.

Nandi S.K. and L.M.S. Palni 1993. Shoot growth and winter dormancy in tea. J. Plantn. Crops. 21 (5): 328-332.

Powell L.E. 1987. Hormonal aspects of bud and seed dormancy in temperate zone woody plants. Hort Sci. 22: 845-850.

Sembdner G. Weiland, J. Aurich, O. and Schreiber K. 1970. Gibberellin glycosides in higher plants: isolation, metabolism and biological acitivity. Zesz Nauk. Uniw. Kopernika w Toruniu, Biol. 12: 191-195.

Schneider G, Schliemann W, Schaller B and Jensen E. 1992. Identification of native gibberellins-O-glucosides in *Zea mays* and *Hordium vulgare.* Progress in Plant Growth Regulation, (Eds: Kerssen CM, van Loon LC and Vreugdenhil, D.) , Dordrecth, Boston, London, Kluwer Acad Pub. 566-570.

Schneider G. and Schliemann W. 1994. Gibberellin conjugates: an overview. Plant Growth Reg. 15: 247-260.

Talla M.C. 1989. Changes in endogenous gibberellins in tulip bulbs induced by different pre-planting treatments. Colture Protette. 18 : 85-88.

Wareing P.F. 1977. Growth substances integration in the whole plant. In : Integration of activity in higher plants. Soc. Exp. Biol. Symp. XXXI. (Ed.: Jenning, D.H.), 337-365.

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